

## Influence of Root-Applied Epibrassinolide and Carbenoxolone on the Nodulation and Growth of Soybean (*Glycine max* L.) Seedlings

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With 3 figures

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### Abstract

24-Epibrassinolide belongs to the brassinosteroid family of plant hormones, and carbenoxolone is a synthetic analogue of glycyrrhizic acid that inhibits enzymes important in steroid and prostaglandin action in animals. The effect of these compounds on root nodulation, root growth and shoot growth of *Glycine max* (L.) Merr. cv. Tracy M was examined. Epibrassinolide reduced total nodulation, plant wet weight, root length, shoot length, first internode length and number of lateral roots. Effects were observed at concentrations as low as 0.1  $\mu$ M. Carbenoxolone reduced lateral root formation, but did not reduce nodule numbers, root length or weight, nor stem length or weight.

**Key words:** *Glycine max* — hormone — lateral root — nodulation — root nodule — soybean

### Introduction

Almost 30 years ago, Mitchell and coworkers isolated an organic compound from *Brassica napus* pollen that promoted stem elongation and cell division in plants (Mitchell et al. 1970). Subsequently, this compound was identified as a steroidal lactone by Grove et al. (1979) and named brassinolide. Since then, over 40 brassinosteroids have been isolated, and brassinosteroids are now thought to represent a ubiquitous class of plant growth regulators (Adam and Marquardt 1986, Mandava 1988, Vardhini and Rao 1998).

Only a few studies have looked at the effect of brassinosteroids on root growth or the effect of root application of these compounds on the growth and development of the whole plant. Roddick and Guan (1991) found that 24-epibrassinolide had a direct inhibitory effect on seedling root development. Also, brassinolide inhibited the formation of adventitious roots in hypocotyls of dwarf bean,

mung bean and cucumber (Adam and Marquardt 1986) and inhibited root growth of cress seedlings (Jones-Held et al. 1996). 24-Epibrassinolide inhibited root elongation in *Arabidopsis thaliana* seedlings (Clouse et al. 1993) and lateral root elongation in maize, mung bean and wheat (Roddick and Ikekawa 1992). Roddick (1994) found that several brassinosteroids inhibited the growth of excised tomato roots. However, root application of brassinosteroids does not always inhibit root growth or seedling development. Ronsch et al. (1993) found that treatment of cuttings of adult Norway spruce with 28-homobrassinolide enhanced the formation of adventitious roots, and Takatsuto et al. (1983) observed that brassinosteroids applied to the roots of young radish or tomato seedlings promoted the elongation of cotyledon hypocotyls and petioles.

Information on the influence of root-applied steroids on the development of soybean plants is limited, and no information exists on the influence plant steroid hormones may have on the nodulation of legumes. The objective of this study was to increase our understanding of these processes. 24-Epibrassinolide (Fig. 1) was applied to soybean roots and the effect on root nodulation by *Bradyrhizobium japonicum*, lateral root formation, root growth and stem growth was monitored. In parallel experiments, the effect of carbenoxolone on the growth and nodulation of soybean plants was monitored. Carbenoxolone (Fig. 1) is an analogue of glycyrrhizic acid which is extracted from the roots of two legumes, *Glycyrrhiza uralensis* and *G. glabra*. Carbenoxolone inhibits enzymes that regulate the concentrations of steroids and prostaglandins in mammals (Baker 1994) and inhibits linoleic acid desaturation in soybean chloroplasts

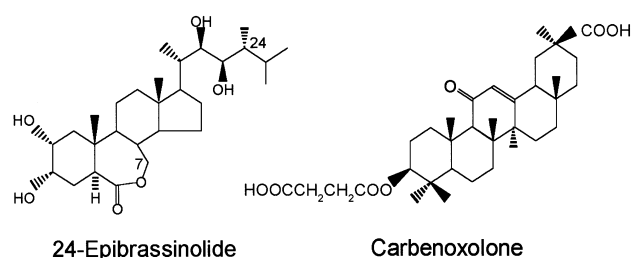


Fig. 1: Structure of 24-epibrassinolide and carbenoxolone

(Norman et al. 1995). Its role in root nodulation and growth has not previously been studied.

## Materials and Methods

### Plant material and growth conditions

Soybean plants [*Glycine max* (L.) Merr. cv. Tracy M, obtained from T. E. Carter, USDA-ARS, North Carolina State University, Raleigh, NC] were grown in a growth chamber under hydroponic conditions. Details on the bacterial inoculum, plant culture and seed treatment are given in Hunter (1983). In brief, seeds were surface-disinfected, placed on agar plates, and germinated in the dark. Four-day-old seedlings were inoculated with nodule bacteria, transferred to hydroponic containers, one plant per container, and placed in a growth chamber. Growth media was one-fifth strength Hoagland's solution containing 2.5 mM potassium nitrate, test compounds, and 0.5 ml of a late-log-phase *B. japonicum* I-110 culture. The growth chamber was operated with a 14-h–30 °C light period ( $340 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR) and a 10-h–20 °C dark period. Humidity was maintained at ~60 % (day) and ~90 % (night). Plants were harvested and measurements made after 21 days of incubation. Each treatment consisted of 20 plants.

### Chemicals

24-Epibrassinolide was provided by Trevor C. McMorris, Department of Chemistry, University of California, San Diego (McMorris 1997). All other chemicals were analytical reagent grade.

### Statistical comparisons

Standard error of the mean computations and statistical comparisons (one-way ANOVA) were performed using the Instat computer program (GraphPad Software Inc., San Diego, CA).

## Results and Discussion

### Studies with epibrassinolide

The addition of epibrassinolide to the rooting medium of soybean seedlings had a strong inhib-

itory effect on root and shoot growth (Fig. 2). After 3 weeks of growth,  $10 \mu\text{M}$  epibrassinolide significantly reduced total plant weight (Fig. 2a,b), root and shoot length (Fig. 2c), first internode length (data not shown), and lateral root and nodule formation (Fig. 2d). At the  $10 \text{ mM}$  epibrassinolide treatment level, plant dry weight was reduced (relative to the untreated controls) by 37 %, plant wet weight by 40 %, root length by 34 %, shoot length by 14 %, first internode length by 27 %, number of lateral roots by 50 % and number of nodules by 69 %. At  $1 \mu\text{M}$  epibrassinolide, root length was reduced by 25 %, lateral root number by 31 % and nodule number by 33 %. At  $0.1 \mu\text{M}$  epibrassinolide, root length was reduced by 30 %, lateral root number by 21 % and nodule number by 36 %. All values were significant at  $P < 0.01$ .

The effect on soybean taproot, lateral root and stem development agrees with results obtained by Roddick and Ikekawa (1992) with hydroponically grown mung bean seedlings but differs from those obtained with maize seedlings, where root-applied epibrassinolide did not affect main root length or lateral root number (Roddick and Ikekawa 1992). Inhibition of taproot elongation and lateral root formation may have been caused by an interaction between the brassinolides and other plant hormones. Brassinolides stimulate ethylene production (Arteca et al. 1985, Schlagnhauer and Arteca 1985) and act in a synergistic manner with auxins to increase their activity (Arteca et al. 1985, Roddick and Guan 1991). Both of these hormones can restrict root growth.

The influence epibrassinolide had on nodulation may be related to its effect on root development. A reduction in taproot length and the number of lateral roots may simply have reduced the number of infection sites available for nodule formation. Alternatively, high levels of epibrassinolide may inhibit nodulation directly or epibrassinolide may interact with other plant hormones to inhibit nodulation. It is unlikely that stimulation of ethylene production was involved in the inhibition of nodulation in these plants as soybeans differ from many other legumes in that ethylene, at physiological concentrations, does not inhibit or regulate nodulation (Hunter 1993, Suganuma et al. 1995, Schmidt et al. 1999). Auxin is required for the formation of soybean root nodules (Fukuhara et al. 1994) and auxin transport inhibitors induce the formation of nodule-like structures in some legumes (Hirsch et al. 1989, Wu et al. 1996). In

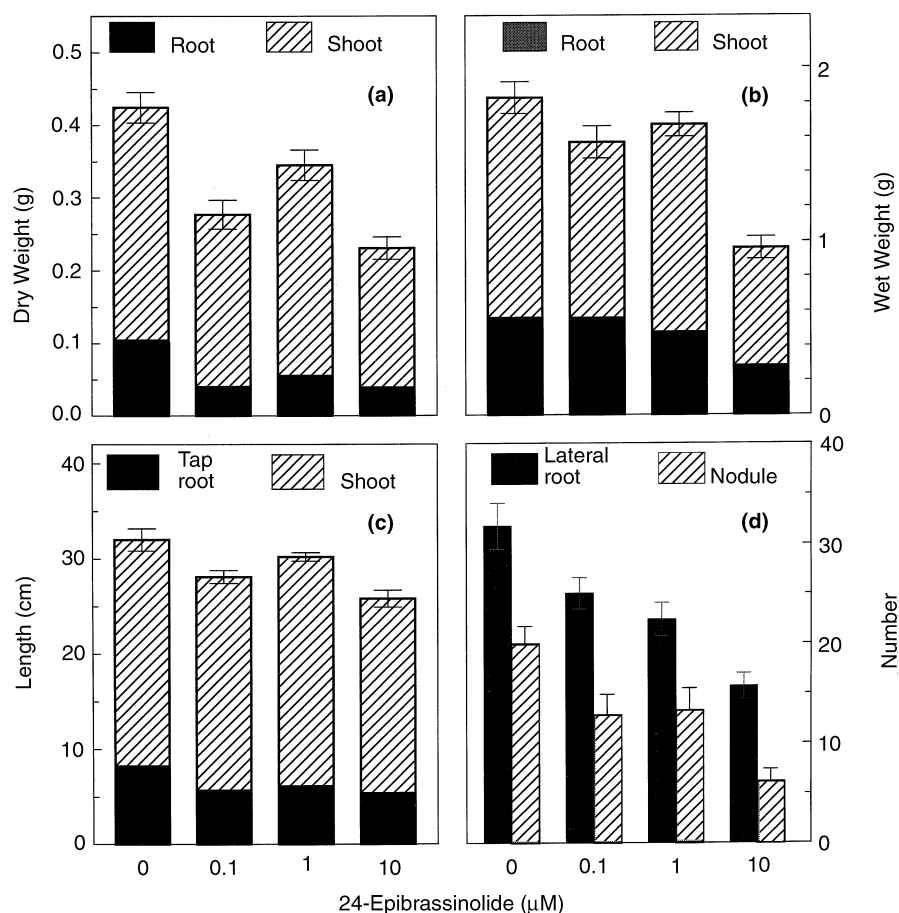


Fig. 2: Effect of 24-epibrassinolide on growth and development of 3-week-old soybean seedlings (mean  $\pm$  S.E.M.; n = 20)

addition, at supraoptimal levels, auxin activity can inhibit root-nodule formation (Kefford et al. 1960, Hunter 1987, Hunter 1989, Fukuhara et al. 1994). Thus, the inhibition of nodulation by epibrassinolide may involve an interaction with auxin or auxin transport.

### Carbenoxolone studies

The presence of carbenoxolone in the growth media at concentrations as high as 500  $\mu\text{M}$  did not significantly alter plant length (data not presented), weight (data not presented), or number of nodules (Fig. 3). However, 500  $\mu\text{M}$  carbenoxolone significantly ( $P=0.0007$ ) reduced the formation of lateral roots (Fig. 3). This suggests an interaction of carbenoxolone with the pathway for growth of lateral roots. At 500  $\mu\text{M}$ , carbenoxolone may be interacting with an enzyme. This would be similar to the mechanism of action of carbenoxolone in animals, in which carbenoxolone inhibits enzymes that inactivate either steroids or prostaglandins (Baker 1994, 1995), which has the effect of raising the local hormone concentration.

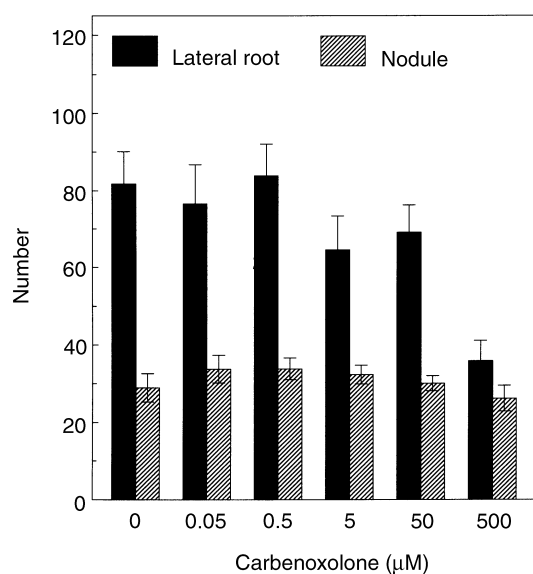


Fig. 3: Effect of carbenoxolone on the formation of lateral roots and root nodules on 3-week-old soybean seedlings (mean  $\pm$  S.E.M.; n = 20)

### Conclusions

Root exposure to epibrassinolide had a negative impact on the overall growth of soybean seedlings.

The strongest effects were on the number of nodules and number of lateral roots that formed. Root exposure to carbenoxolone was found to reduce the number of lateral roots that formed.

## Zusammenfassung

### Einfluss von auf die Wurzel applizierten Epibrassinolids und Carbenoxolons auf die Knöllchenbildung und das Wachstum von Sojabohnen (*Glycine max* L.)-Sämlingen

24-Epibrassinolid gehört zu der Brassinosteroid-Familie von Pflanzenhormonen und Carbenoxolon und ist ein synthetisches Analog von Glycerinsäure, die Enzyme inhibieren, die hinsichtlich der Steroid- und Prostaglandinwirkungen bei Tieren. Der Einfluss dieser Verbindungen auf die Knöllchenbildung an Wurzeln, das Wurzelwachstum und das Sprosswachstum von *Glycine max*. (L.) Merr. cv. Tracy M wurde untersucht. Epibrassinolid reduzierte die Gesamtknöllchenbildung, das Pflanzenfrischgewicht, die Wurzellänge, die Sprosslänge, Länge des ersten Internodiums und Anzahl der Seitenwurzeln. Die Wirkungen wurden bereits bei geringen Konzentrationen wie 0.1  $\mu\text{M}$  beobachtet. Carbenoxolon reduzierte die Bildung von Seitenwurzeln, hatte aber keine Wirkung hinsichtlich einer Reduzierung der Anzahl der Knöllchen, der Wurzellänge oder des Wurzelgewichts sowie der Stengelgröße oder Stengelgewichts.

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